formational change are the subject of detailed ongoing studies, the crosslinking and late addition cosolvent clearly limit perturbation to the protein structure and to the crystal. How limited are conformational changes in the crystalline environment? In general, only slight changes in protein conformation are allowed that are distant from crystal contacts and do not damage the crystal lattice. Nevertheless, a number of investigators were able to interpret small changes in crystal structures as a function of temperature and pressure in terms of protein function and folding (Urayama et al., 2002; Jacob et al., 1998). Additionally, significant progress has been made in the structural characterization of protein catalytic cycles that include limited conformational changes (Petsko and Ringe, 2000). The timescale and to some extent the nature of the conformational changes are, however, expected to be affected by the crystal contacts (Zhu et al., 1992). Because the majority of the protein is restrained in the native structure, the strategy presented by Buhrman et al. has the advantage that conformational effects of the cosolvent are likely to be limited to regions of the protein that remain flexible in the crystal, such as loops or residues in active site clefts.

A critical aspect of the study by Mattos and coworkers is the clear demonstration that the cosolvent-stabilized structure is functionally relevant. Significant effects of the cosolvent on the structure are confined to the switch II region. Furthermore, the structure that is stabilized is closely identical to that seen in Ras when bound to its regulatory or effector proteins. Only few reports exist where a relationship between cosolvent-stabilized structure and function has been demonstrated as clearly (Buck, 1998). This relationship is consistent with the general observation that alcohol-based cosolvents are not indiscriminate in their effect but that the structures that are stabilized reflect intrinsic conformational preferences of the polypeptide chain. It is likely that such alternative conformations of the protein have been preserved in evolution for the purpose of folding or function. An increased repertoire of solution conditions for crystallization trials and the ability to explore the flexibility of proteins in crystals by the use of alcohol cosolvents and crosslinking, as proposed here, will allow us to study different conformational states of protein segments. The automation that is possible will provide many high-resolution structures as part of this strategy. Homology modeling and other computational techniques will, in conjunction with additional experiments, suggest the functional importance of the conformational states thus sampled.

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Structure, Vol. 11, July, 2003, ©2003 Elsevier Science Ltd. All rights reserved. DOI 10.1016/S0969-2126(03)00129-1

Mycobacterial ABC Transport System: Structure of the Primary Phosphate Receptor

The crystal structure of the mycobacterial PstS-1, the primary phosphate receptor of the ABC transport system, reveals a number of features that account for the tight binding and high specificity along with the close similarity to the phosphate binding protein of *Escherichia coli.*

The capture of essential small molecules and their transport into microorganisms is a fertile area of research, particularly with novel information coming out on ABC transport systems, such as that for phosphate (Torriani, 1990). ABC-type transport systems are responsible for importing a variety of small molecules such as various essential amino acids, sugars, and anions.

The binding proteins that act as initial receptors for such systems in Gram-negative bacteria are well understood in terms of their ligand binding and structures (Quiocho, 1991). These proteins may be expressed in response to starvation for the particular essential molecules. In *Escherichia coli* and other Gram-negative bacteria, an N-terminal peptide directs the protein to the

periplasm, before being cleaved from the mature protein at the membrane. The binding proteins for different ligands generally share little in sequence similarity but a considerable amount in overall three-dimensional structure. Specifically, they have a ligand binding cleft between two globular domains, and this cleft closes around the bound ligand through a hinge-bending movement of the domains. These proteins are typically very specific for binding their ligand. Once the binding proteins have scavenged for low concentrations of their specific ligand, the ligand is transferred from these initial receptors to membrane proteins for import into the cytoplasm.

Apart from the inherent interest in transport systems and the potential for drug targeting, these binding proteins have also been the subject of detailed studies of how specific binding occurs when no enzymatic activity is required of the binding site. In addition, the proteins have been used in technical developments in biomedical research. For example, either the signal N-terminal peptide or the whole protein has been exploited to form a fusion protein expression system that might result in the expressed protein of choice being exported to the periplasm for easy purification. The binding proteins have also been used in biosensor development (Brune et al., 1994; Gilardi et al., 1994; Marvin et al., 1997). The tight binding and high specificity of the ligand has led to their use as frameworks for protein-based sensors, particularly using fluorescence as the method of measurement.

Mycobacteria tuberculosis is Gram positive and its ABC transport systems involve an alternative strategy with the initial substrate receptor tethered to the outer surface of the membrane (Braibant et al., 2000). A 38 kDa membrane-attached protein was identified in this organism with 30% sequence identity to the phosphate binding protein of *E. coli*. It was initially identified because it is strongly immunogenic and its presence is sensitive to phosphate starvation. This protein, PstS-1 (phosphate-specific transport substrate binding protein 1) was presumed to play a role in phosphate capture for subsequent import. Because of the major disease implications of this mycobacterium, characterization of the protein may have significance in vaccine or diagnostic development.

The structure of this PstS-1 protein with a bound phosphate anion has now been determined to atomic resolution, presented in this issue of *Structure* (Vyas et al., 2003). It is the first structure of such a membrane-tethered ligand receptor. It has interesting similarities to, but significant differences from, the phosphate binding protein of *E. coli*. Several structures of the latter protein have been solved, including both phosphate-bound and phosphate-free forms (Ledvina et al., 1996; Luecke and Quiocho, 1990). These revealed the details of the domain movements that result in completely burying the bound phosphate. Overall, phosphate binds in a cleft between two domains similarly for the proteins from *E. coli* and *M. tuberculosis*. In addition, PstS-1 has a flexible extension at the N terminus consisting of 26 amino acids. This extension anchors it to the cell surface via a covalently attached lipid group. Its flexibility presumably also allows the domain movements essential for tight phosphate capture, although the protein is kept close to the membrane surface.

The structure of the new protein reveals much about the interactions between this simple anion and amino acids in the binding site. The tetrahedral inorganic phosphate is bound to the protein without any water molecules bound to the phosphate. The four oxygens of the phosphate are anchored by 13 hydrogen bonds, including 11 where an NH or OH group provides the hydrogen. All but two of these 13 are conserved in the phosphate binding protein, which has 12 hydrogen bonds in total. Of the two not conserved, one hydrogen bond is lacking altogether and the amino acid in the other bond is different.

Both PstS-1 and phosphate binding protein bind phosphate quite tightly over a wide pH range where either $H_2PO_4^-$ or HPO_4^{2-} is the form that dominates in solution. Significantly, PstS-1 has two aspartates in the binding site that can act as hydrogen bond acceptors. This ensures its ability to bind tightly the $H_2PO_4^-$ form that predominates in the pH region below 7. In contrast, the phosphate binding protein has only one aspartate, the other aspartate position being taken by a threonine, potentially a disadvantage below pH 7. In both proteins the aspartate(s) is important for specificity, such as for over the similarly tetrahedral sulfate. That anion cannot provide a hydrogen atom for bonding to an aspartate at normal pH. The extra aspartate in *M. tuberculosis* may well contribute to the ability of this organism to scavenge phosphate efficiently in acidic conditions. Such a feature in these receptors may be important for the ability of mycobacteria to survive at low pH.

Although the binding site is for an anion, the structure shows that the cleft has a negatively charged electrostatic surface. Thus, the specific ion-dipole interactions outweigh overall charge considerations. A question remains as to why the protein has this feature given that it presumably reduces affinity for its ligand. However, it needs to be borne in mind that the biological function also may require specific exit of the phosphate to allow eventual entry into the cell. Tight binding under conditions of low phosphate concentrations is only one aspect of these proteins' function.

The large involvement of hydrogen bonding and the lack of hydration seem to be shared features in the association of ligand with such binding proteins along with the binding cleft closed by the hinge bend of two domains (Quiocho, 1991). The directionality and specificity of hydrogen bonds may aid the tight binding and overall specificity of the ligand-protein interaction.

This structure therefore gives us a picture of how an array of hydrogen bonds is important for the specific and tight capture of inorganic phosphate. The two aspartates provide a mechanism for the binding at low pH, where this bacterium is known to survive. The structure also shows how the successful method of binding is shared with the phosphate binding protein of Gram-negative bacteria.

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Structure, Vol. 11, July, 2003, DOI 10.1016/S0969-2126(03)00132-1

Calcium-Driven Changes in S100A11 Structure Revealed

The structure of apo-S100A11 reveals the key role of calcium-induced conformational change in triggering the release of the binding site for phospholipid binding protein annexin I.

The S100 proteins are a major subfamily of EF-hand calcium sensor proteins. Many of the ~20 S100s identified so far have been associated with multiple targets that promote cell growth and differentiation, cell cycle regulation, transcription, and cell surface receptor activities (Donato, 2001). The detection of abnormal S100 gene regulation in different disease states including tumor progression, Alzheimer's, cardiomyopathy, psoriasis, and chronic inflammation has fueled further clinical interest in this multigenic family of proteins (Heizmann et al., 2002). Notable features of S100 proteins are high sequence homology in their Ca^{2+} binding loops, a distinctive dimeric architecture, and a unique N-terminal EF hand. Though all form homodimers, some S100s are known to preferentially form heterodimers, such as S100A8/A9 and S100A1/B.

Calcium binding triggers a conformational change in the EF hands that exposes a hydrophobic surface in each subunit (Fritz and Heizmann, 2003), composed largely of residues from helices III and IV and the linker region. Typically, the binding site on the target is a short helical segment rich in basic and hydrophobic residues. In theory, the two binding sites per S100 dimer can bind similar or different target molecules. Furthermore, the target peptide can occupy different orientations within the binding site in accord with the distinctive hydrophobic character and charge distributions seen in structures of S100B bound to p53, CapZ, and NDR kinase. The response to calcium is not the only factor that influences the binding of targets to S100 proteins. Interactions may be fine-tuned further by the binding of zinc and copper ions. Recent crystallographic evidence from the S100A12 hexamer also supports the existence of alternate oligomerization states (Moroz et al., 2001). An oligomerization-based model was proposed as a mechanism for the signaling of the multimeric receptor for advanced glycation end products (RAGE). Another alternate structural state arises from the oxidation of S100B into a disulfide crosslinked form that promotes neurotropic effects in the extracellular space (Donato, 2001).

Several S100s play a crucial role in cytoskeleton development and assembly through interactions with annexins in a Ca^{2+}-dependent manner during endo- and exocytosis of cells (Gerke and Moss, 2002). The conserved core domain of the annexins binds to membrane phospholipids in a Ca^{2+}-dependent manner, an event that is modulated by interactions of the variable N-terminal domain with partner proteins such as S100s. The X-ray structure of N-terminal peptides of annexin II complexed with S100A10 (p11; Rety et al., 1999) and of annexin I with S100A11 (S100C; Rety et al., 2000) provide a structural basis for understanding the specificity of S100-annexin interactions. Cryoelectron microscopy has helped recreate the higher order arrangements of the S100A10/annexin II complex in the membrane environment, but more recently the very first structure of intact annexin I has provided insight into the events that lead to membrane fusion by Ca^{2+}/S100A11 binding. It is now known that Ca^{2+} binding releases the N-terminal α helix of annexin I from the core domain of the protein, enabling interactions with membrane or S100A11 (Rosengarth et al., 2001).

The present model for membrane fusion is based on the linking of charged membrane surfaces by two molecules of annexin I core domain brought together through binding to one S100A11 dimer. In view of these studies, the solution NMR structure of apo-S100A11 in this issue of *Structure* by Shaw and coworkers (Dempsey et al., 2003) reveals critical atomic details of the binding site in the absence of annexin I. In particular, this new structure reveals the extensive changes induced by Ca^{2+} binding that transforms the closed binding site into a target ready state. This is important because the only other S100/annexin complex involves S100A10, which does not contain functional Ca^{2+} binding sites and has evolved a permanently "open" conformation resulting in constitutive binding to annexin II.

Comparisons of apo-S100A11 and the S100A11/annexin I complex reveal some differences in the dimer